

DROSOPHILA PSEUDOOBSCURA (DIPTERA: DROSOPHILIDAE)
OF THE GREAT BASIN IV: A RELEASE EXPERIMENT AT BRYCE CANYON

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ABSTRACT.—Some populations of *Drosophila pseudoobscura* in the Great Basin have very little genetic variation for third chromosome inversion gene arrangements. These populations are essentially monomorphic for the Arrowhead gene arrangement. At Bryce Canyon, Utah, individuals with other gene arrangements (Standard, Pikes Peak, and Treeline) were released and their frequencies monitored. One generation after release, the released arrangements had increased in frequency from 0.7% to almost 10%. After overwintering, the arrangement frequencies were not statistically different from the prerelease samples. The samples did demonstrate a low-level retention of the released Pikes Peak arrangement. The decline in the released arrangements was probably the result of large population size at Bryce Canyon and the bottleneck effects of overwintering. The results do not seem consistent with a model of the released arrangements having a lowered fitness.

Natural populations of *Drosophila pseudoobscura* have been studied for over 40 years. Populations were originally cytologically characterized for the gene arrangements of the third chromosome (Dobzhansky and Sturtevant 1938), and these arrangement frequencies were monitored. The different gene arrangements are the result of a phylogeny of mostly overlapping inversions of segments of the third chromosome (Dobzhansky 1944). Because of greatly reduced recombination within the inverted segments in heterokaryotypes, these inversions act to tie together portions of the chromosome into large supergenes. These supergenes (gene arrangements) are inherited and behave in populations as alleles at a single locus. Some natural populations of *D. pseudoobscura* have been sampled repeatedly since the 1930s (Anderson et al. 1975) and provide an excellent historical basis for current research. These continuing geographic surveys over the range of *D. pseudoobscura* have led to the division of the species into five geographic races, each characterized by the gene arrangements present and their frequencies (Dobzhansky and Powell 1975). These races are:

1. Pacific Coast
2. Intermountain Plateau
3. Rocky Mountains and Texas
4. Northern Mexico
5. Southern Mexico and Guatemala

Race 2, the Intermountain Plateau, has been

characterized as nearly monomorphic for the Arrowhead (AR) gene arrangement, while the other races are highly polymorphic.

Populations of the Intermountain Plateau were originally sampled and characterized in the early 1940s. Those areas which have been sampled regularly (Bryce Canyon, Utah; Lehman Caves, Nevada; and three localities in Arizona) have remained essentially the same as the original samples (Anderson et al. 1975). During 1976 and 1977 six areas in the northern Intermountain Plateau area were sampled; some of these sites had not been sampled since they were originally characterized in the 1940s and 1950s. This area, which had previously been characterized as nearly monomorphic AR, had undergone great changes both in the particular gene arrangements present and their frequencies (Turner and Jeffery 1980). These populations now have an arrangement array very similar to that found in the Rocky Mountain populations (Race 3) including the endemic Fort Collins arrangement, previously found only in a few areas of the Rocky Mountains. It was hypothesized that these changes came about because of gene flow into these northern Intermountain Plateaus (Race 2) from the Rocky Mountains (Race 3) (Turner and Jeffery 1980).

The release of genes into natural populations has been attempted previously with positive results. Bryant (1976) released *D. pseudoobscura* with a rare esterase allozyme into a

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small oasis population in Death Valley, California, in an attempt to swamp the population and then determine the amount of immigration in the population. Thirty-eight days after the release, the frequency of the genetic marker had increased from 2% before the release to approximately 80%. Dubinin and Timiakov (1946) released *D. funebris* with an inversion from another locality into a population where that inversion was rare. The frequency of the released inversion was originally 0.35% and increased to a high of 49.5% approximately one generation after the release. These two results show that it is possible to introduce outside genes or gene arrangements (even those that have been in the laboratory for some time) into a natural population and that they can become integrated into that population at fairly high frequencies.

This research investigates, through the use of a release experiment, the inversion gene arrangement polymorphism in an Intermountain Plateau population of *Drosophila pseudoobscura*.

MATERIALS AND METHODS

SAMPLING SITES AND METHODS.—Three locations in Bryce Canyon National Park, Utah, were selected as sampling sites. These three sites are separated by distances such that migration by individuals between the sites was thought to be negligible. The southern and northern sampling sites are 14 km apart. The middle site is 5 km from the southern site and 9 km from the northern site. These three areas are quite diverse and probably encompass the entire range of possible *D. pseudoobscura* habitats in Bryce Canyon. The southern sampling site is the least arid and the most similar of the three sites to the northern Intermountain Plateau. Because of this similarity, this site was chosen for the release. During 21–28 June 1978, samples were obtained from each of these three sites. Individual females obtained from nature were used to establish isofemale lines, and F1 larvae were characterized cytologically for the gene arrangement of the third chromosome using standard *Drosophila* salivary techniques.

THE RELEASE.—On the three days immediately following the completion of the initial samples, *D. pseudoobscura* homozygous for Standard (ST), Treeline (TL), or Pikes Peak

(PP) gene arrangements were released in the southern sampling area. The flies to be released were grown in large population cages which support approximately 12,000 adult flies. The releases were accomplished by opening these cages and allowing the adults to escape. Thus the released flies were a mixed sample of age and sex, and no attempt was made to release only virgin flies. This was done in five separate release events over the span of three days, one release daily during the evening activity period and on two days releases during the morning activity periods. Approximately equal numbers (approx. 12,000) of each homozygous type were released during each event, and the total number of individuals released was approximately 200,000. No flies were released in the other two sampling sites. All three sites were subsequently sampled and characterized genetically during August 1978 and June, August, and September 1979.

ORIGIN OF RELEASED CHROMOSOMES.—The released PP stock was derived from a collection made in June 1977 at American Fork, Utah. In this collection PP had a frequency of 18.9%. The TL stock came from a collection in Big Cottonwood Canyon, Utah (near Salt Lake City), in June 1977. The frequency of TL in this population was 25.0% (Turner and Jeffery 1980). The ST stocks were from Mather, California, and have been maintained in the laboratory since 1959.

RESULTS AND DISCUSSION

The frequencies of the third chromosome gene arrangements from the initial sample (June 1978) are given in Table 1 along with the totals from previous samples of Bryce Canyon. The June 1978 totals are significantly different from the previous samples total ($\chi^2 = 13.45$, $p < .01$, 3 df) but not significantly different from the latest (1973) sample of Bryce ($\chi^2 = 1.51$, $p < .25$, 1 df). The three sample sites have no significant differences in gene arrangement frequencies (Table 1) ($\chi^2 = 1.4$, $p < .79$, 2 df). The June 1978 samples had two arrangements, Treeline (TL) and Bryce (BR), not previously found in Bryce Canyon samples. The BR arrangement is a new arrangement, an inversion of Arrowhead (AR) with breakpoints 71B and 80C.

The release took place after the June 1978

TABLE 1. Percentage of third chromosome gene arrangements of *D. pseudoobscura* from Bryce Canyon before the release (n = number of chromosomes).

Site	Sampling date	AR	PP	CH	TL	ST	BR	n
Bryce Canyon	1940	96.0	—	2.0	—	2.0	—	100
	1950	92.9	—	2.4	—	4.8	—	84
	1957	93.2	2.6	1.6	—	2.6	—	190
	1965	92.0	1.5	4.0	—	2.5	—	200
	1973	99.3	—	—	—	.7	—	136
(Anderson et al. 1975)								
Totals		94.6	1.1	2.1	—	2.1	—	710
Bryce Canyon								
Northern site	June 1978	97.8	—	2.2	—	—	—	178
Middle site	June 1978	97.0	—	1.8	0.3	1.0	—	398
Southern site	June 1978	98.4	—	1.0	—	0.3	0.3	304
Totals	June 1978	97.6	—	1.6	0.1	0.6	0.1	880

TABLE 2. Percentage of third chromosome gene arrangements of *D. pseudoobscura* in postrelease samples from Bryce Canyon, Utah (August 1978) (n = number of chromosomes).

Locality	Days since release	AR	CH	ST	PP	TL	SC	n
Northern	day 42 & 43	96.8	2.4	0.8	—	—	—	126
Middle	day 42 & 43	96.0	2.5	0.5	0.5	0.5	—	202
Southern	day 40	97.1	—	1.0	1.0	—	1.0	102
	day 41	96.8	1.1	—	—	2.1	—	94
	day 42	90.5	—	1.2	3.6	4.8	—	84

samples and only in the southern site. The populations were sampled again in August 1978 (Table 2). While the nonrelease sites have not changed significantly, the daily gene frequencies from the southern area show the release to have been successful. On days 40 and 41 (since the release), the frequencies (Table 2) are similar to the prerelease samples, but on day 42 the frequency of AR decreased from over 96% the previous day to 90.5%. This decrease in frequency is due to the increased frequencies of the released TL and PP gene arrangements. Again, this decreased AR is not seen in either of the two nonrelease sites even though they were sampled past day 41. These frequency increases are the result of the emergence of the F1 from the released individuals. These data show the released chromosomes (ST, TL, and PP) to have increased from 0.33% in the initial samples to almost 10% 42 days after the release. This appearance 42 days after the release indicates at Bryce Canyon the generation time of 42 days. At this length, these populations could have only three or four generations a year.

From September until April or May temperatures at Bryce Canyon are too cold to support an active *D. pseudoobscura* population. Thus, the next samples following the release were during the summer 1979. Three collections were made, and the gene arrangement frequencies are presented in Table 3. There are no statistically significant differences between samples or sampling sites ($X^2 = .74$, $p < 0.6$, 2 df). Pikes Peak (PP) was the only gene arrangement not seen in the prerelease samples but found in the 1979 samples. Although a very large number of individuals were released, the resulting change in gene frequencies was small. Reduced survival of the released individuals probably was responsible for a portion of the decline. Dobzhansky and Wright (1943) calculated the survival of released 5–7 day-old orange-eyed *D. pseudoobscura*. They estimated that approximately 90% survive per day. That is probably an overestimate for the current release since some of the released flies were older (and younger) than their samples. Also, Bryce Canyon is 3,000 ft higher in altitude than Dobzhansky and Wright's experimental

TABLE 3. Percentage of third chromosome gene arrangements from sites in Bryce Canyon for summer following August 1978 release (n = number of chromosomes).

Date	Site	AR	CH	ST	PP	n
June 1979	Northern	94.1	—	4.4	1.5	68
	Middle	98.3	0.9	0.9	—	116
	Southern	98.0	1.5	0.5	—	204
	Total	97.4	1.0	1.3	0.3	388
Aug. 1979	Northern	97.2	1.7	1.2	—	176
	Middle	100.0	—	—	—	50
	Southern	97.9	2.1	—	—	144
	Total	97.8	1.6	0.5	—	370
Sept. 1979	Northern	96.2	2.8	—	0.9	106
	Middle	100.0	—	—	—	40
	Southern	93.9	3.8	1.5	0.8	130
	Total	95.7	2.9	0.7	0.7	276
1979	Total	97.1	1.7	0.9	0.3	1034

location. In fact, in their study, when the weather was particularly arid, their recapture rates declined significantly. The increased altitude of Bryce Canyon, with reduced humidity and greater temperature extremes, probably contributes to a survival rate lower than their 90% per day estimate. But, more importantly, in the current release the concern is not on the survival of an individual but the genetic contribution to the next generation. A released female may survive, but unless she can find a suitable oviposition site and sufficient food, she will not contribute genetically to the next generation and the genes she carried will be lost. Laboratory experiments with *D. pseudoobscura* females show over 90% reduction in productivity (number of offspring) under nutritional stress (Turner and Anderson 1983). For these reasons the contributions of released females were probably small in magnitude. In all cases the released chromosomes in the second sample were found as heterozygotes. The contribution of already-mated released females would have been F1 homozygotes. Since virgin females were not released and no homozygotes were found, probably most of the released chromosomes that became integrated into the gene pool were the result of released males mating with wild (AR/AR) females. In retrospect, for a population like Bryce Canyon (one very close to the ecological bounds of the species) a release of males only may be the best strategy.

In the prerelease samples, none of the

three sampling sites shows significant differentiation. This lack of spatial differentiation between sites is surprising considering the ecological difference between sites. For instance, only ponderosa pine (*Pinus ponderosa*) is found in all three sampling sites, and it is rare in the southern site. There is also a drop in altitude from the southern site (8,300 ft) to the northern site (7,200 ft). These results may indicate that the Arrowhead arrangement (AR) is responding to some general component of the environment such as temperature. If it were responding to something specific in the environment, different sites with different habitats would be expected to show different frequencies. The homogeneity between sample sites is consistent with the observation that much of the Great Basin *D. pseudoobscura* populations are characterized by a high frequency of the AR arrangement (Anderson et al. 1975, Dobzhansky and Powell 1975).

One other interesting aspect of these samples (August 1978, Table 2) is the appearance of the Santa Cruz (SC) gene arrangement at Bryce Canyon. This arrangement was not in the release, and its appearance at this time is only coincidental. This arrangement is usually found along the Pacific Coast of California and in southern Mexico. In previous samples of almost 1,600 chromosomes (Table 1) SC had never been identified at Bryce. In samples from related areas (Nevada, Arizona, and Utah) (Anderson et al. 1975, Turner and Jef-

fery 1980), nearly 5,000 chromosomes have been characterized and SC has never been identified in this region. Whether SC has always been at Bryce but in extremely low frequency or whether this SC came with an individual immigrant (active or passive) fly from a population where the SC arrangement is found cannot be determined at this time, although the latter seems the more probable alternative.

TL, which had reached levels close to 5% in the southern sampling area 42 days following the release (Table 2), was not observed in over 1,000 chromosomes examined in 1979, of which almost 500 came from the southern (release) area (Table 3). A second gene arrangement in the release, Standard (ST), did not show a significant increase in postrelease samples (Table 2) and had approximately the same frequency in 1979 (Table 3) as that seen in the 1978 prerelease samples (Table 1). In the June 1979 sample from the northern site, Standard has a frequency of 4.4% (Table 3), but it decreased and was not found in the August 1979 sample from this site. Standard did not show a frequency increase in the release area (Tables 1, 2). The observation from the northern site could be the result of migration of the released flies (or their offspring) to the northern site or the result of sampling error due to small sample sizes. The results of these two arrangements (TL and ST) are similar and consistent with no long-term effect of the release.

The PP gene arrangement had not been found in Bryce since 1964, but it did reach a level of 3.6% following the release (Table 2) and was found in low frequency (0.3%) in 1979. During the 1979 samples PP was found in all three sample sites and is most probably a low-level retention following the incorporation of the released PP arrangement into the Bryce Canyon gene pool. This result would indicate that the earlier assumption that the sites were far enough apart to ignore migration was incorrect. In the two to three generations since the release all three sites have two (PP and ST) of the three released arrangements, a linear distance of 14 km. With this level of migration we can consider this area as supporting one large breeding population. In this large population an introduced gene (released or migrant) may be swamped by the local (AR) gene pool, without being able to

enter a small isolate and increase in frequency (as the result of either stochastic or selective mechanisms). This type of population structure would minimize the effects of migration in changing gene frequencies.

Additionally, to persist in this population, an arrangement (or gene) must survive the winter. When high-elevation populations overwinter, they undergo a severe bottleneck in population size because of the low temperatures. How *D. pseudoobscura* overwinter is not known, but overwintering is unquestionably a severe process. In laboratory experiments on overwintering ability only a small frequency of the flies survive cold shocks of just a few days (Jefferson et al. 1974), and over a winter of six to eight months only a very small number of individuals may survive. In this way, the released arrangements may not survive the winter and the sampling effect of the resultant population bottleneck. This bottleneck would eventually cause the population to lose variability through genetic drift. These results also bear on the question of overwintering; that is, whether these higher-altitude populations overwinter in place (at altitude) rather than be repopulated each spring from lower-altitude overwintering refugia. The latter hypothesis is similar to that observed in the desert populations of *D. pseudoobscura* where populations are refounded each year from neighboring mountains after the hot summers have eliminated the populations (Jones et al. 1981). The persistence of the Pikes Peak (PP) arrangement would be consistent with an overwintering-in-place hypothesis and not a repopulation each spring from lower-altitude populations.

A decrease in frequency due to small fitness differences would take many generations. The PP and TL arrangements were isolated from areas with an environment and elevation roughly similar to Bryce Canyon. Both arrangements were in relatively high frequency where found originally, PP being almost 20% and TL 25%. Thus, if fitness differences do exist between the released chromosomes and the native AR, they most likely would be small. Since *D. pseudoobscura* do not reproduce during the winter, from August 1978 until June 1979 there is only one generation and at most two. The rapidity of the decrease would suggest that if the decrease were due to an inherent selective disadvantage, this fit-

ness difference must be extremely large. The rapid decrease would seem more consistent with the swamping effect of a large population size and random drift in overwintering populations.

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